



09/19/00

UTILITY PATENT APPLICATION TRANSMITTAL <small>(Only for new nonprovisional applications under 37 CFR 1.53(b))</small>	Attorney Docket No.	7732-020-27 DIV
	First Inventor or Application Identifier	MICHAEL CLIMO, ET AL.
	Title	COMPOSITIONS AND METHODS FOR TREATMENT OF STAPHYLOCOCCAL INFECTION WHILE SUPPRESSING FORMATION OF ANTIBIOTIC-RESISTANT STRAINS

JC760 U.S. PTO

09/665077



09/19/00

APPLICATION ELEMENTS <small>See MPEP chapter 600 concerning utility patent application contents</small>	ADDRESS TO: Assistant Commissioner for Patents Box Patent Application Washington, DC 20231
1. <input checked="" type="checkbox"/> Fee Transmittal Form (e.g. PTO/SB/17) <small>(Submit an original and a duplicate for fee processing)</small> 2. <input checked="" type="checkbox"/> Specification Total Pages 15 3. <input type="checkbox"/> Drawing(s) (35 U.S.C. 113) Total Sheets <input type="text"/> 4. <input checked="" type="checkbox"/> Oath or Declaration Total Pages 3 a. <input type="checkbox"/> Newly executed (original or copy) b. <input checked="" type="checkbox"/> Copy from a prior application (37 C.F.R. §1.63(d)) <small>(for continuation/divisional with box 15 completed)</small> i. <input type="checkbox"/> DELETION OF INVENTOR(S) Signed statement attached deleting inventor(s) named in the prior application, see 37 C.F.R. §1.63(d)(2) and 1.33(b). 5. <input checked="" type="checkbox"/> Incorporation By Reference <small>(usable if box 4B is checked)</small> The entire disclosure of the prior application, from which a copy of the oath or declaration is supplied under Box 4B, is considered to be part of the disclosure of the accompanying application and is hereby incorporated by reference therein.	ACCOMPANYING APPLICATION PARTS 6. <input type="checkbox"/> Assignment Papers (cover sheet & document(s)) 7. <input type="checkbox"/> 37 C.F.R. §3.73(b) Statement <input type="checkbox"/> Power of Attorney <small>(when there is an assignee)</small> 8. <input type="checkbox"/> English Translation Document <small>(if applicable)</small> 9. <input type="checkbox"/> Information Disclosure Statement (IDS)/PTO-1449 <input type="checkbox"/> Copies of IDS Citations 10. <input checked="" type="checkbox"/> Preliminary Amendment 11. <input checked="" type="checkbox"/> White Advance Serial No. Postcard 12. <input type="checkbox"/> Small Entity Statement(s) <input checked="" type="checkbox"/> Statement filed in prior application. Status still proper and desired. 13. <input type="checkbox"/> Certified Copy of Priority Document(s) <small>(if foreign priority is claimed)</small> 14. <input type="checkbox"/> Other:
15. If a CONTINUING APPLICATION, check appropriate box, and supply the requisite information below: <input type="checkbox"/> Continuation <input checked="" type="checkbox"/> Divisional <input type="checkbox"/> Continuation-in-part (CIP) of prior application no.: 09/263,776, Filed MARCH 5, 1999 Prior application information: Examiner: BORIN, M. Group Art Unit: 1631	
16. Amend the specification by inserting before the first line the sentence: <input checked="" type="checkbox"/> This application is a <input type="checkbox"/> Continuation <input checked="" type="checkbox"/> Division <input type="checkbox"/> Continuation-in-part (CIP) of application Serial No. 09/263,776 Filed on MARCH 5, 1999 <input type="checkbox"/> This application claims priority of provisional application Serial No. Filed	
17. CORRESPONDENCE ADDRESS Steven B. Kelber PIPER MARBURY RUDNICK & WOLFE LLP 1200 Nineteenth Street, N.W. Washington, D.C. 20036-2412 Telephone No. (202) 861-3900 Facsimile No. (202) 223-2085	

Name:	Steven B. Kelber	Registration No.:	30,073
Signature:		Date:	9-19-2000
Name:	Laura D. Nammo	Registration No.:	42,024

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

INVENTOR(S) MICHAEL CLIMO, ET AL.

SERIAL NO: NEW DIVISIONAL APPLICATION

FILING DATE: HEREWITH

FOR: COMPOSITIONS AND METHODS FOR TREATMENT OF STAPHYLOCOCCAL INFECTION WHILE SUPPRESSING FORMATION OF ANTIBIOTIC-RESISTANT STRAINS

FEE TRANSMITTAL

ASSISTANT COMMISSIONER FOR PATENTS
WASHINGTON, D.C. 20231

FOR	NUMBER FILED	NUMBER EXTRA	RATE	CALCULATIONS
TOTAL CLAIMS	16 - 20 =	0	× \$18 =	\$0.00
INDEPENDENT CLAIMS	2 - 3 =	0	× \$78 =	\$0.00
<input type="checkbox"/> MULTIPLE DEPENDENT CLAIMS (If applicable)			+ \$260 =	\$0.00
<input type="checkbox"/> LATE FILING OF DECLARATION			+ \$130 =	\$0.00
BASIC FEE				\$690.00
TOTAL OF ABOVE CALCULATIONS				\$690.00
<input checked="" type="checkbox"/> REDUCTION BY 50% FOR FILING BY SMALL ENTITY				\$345.00
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- ☐ Please charge Deposit Account No. 50-1442 in the amount of . A duplicate copy of this sheet is enclosed.
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Respectfully submitted,

PIPER MARBURY RUDNICK & WOLFE LLP

Laura D. Nammo

Steven B. Kelber

Registration No. 30,073

Laura D. Nammo

Registration No. 42,024

Date: 9-19-2000

1200 Nineteenth Street, N.W.
Washington, D.C. 20036-2412
Telephone No. (202) 861-3900
Facsimile No. (202) 223-2085

COPYApplicant or Patentee: Michael Climo, et al.Serial or Patent No.: _____ Atty. Dkt. No.: 7732-014-27Filed or Issued: March 5, 1999For: Compositions and Methods for Treatment of Staphylococcal Infection While Suppressing Formation of Antibiotic-Resistant Strains**VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY STATUS****(37 CFR 1.9(f) and 1.27(c)) – SMALL BUSINESS CONCERN**

I hereby declare that I am

() the owner of the small business concern identified below:

(XX) an official of the small business concern empowered to act on behalf of the concern identified below:

NAME OF CONCERN AMBI, INC.ADDRESS OF CONCERN 4 Manhattanville Road, Purchase, New York 10577-2197

I hereby declare that the above identified small business concern qualifies as a small business concern as defined in 13 CFR 121.3-18, and reproduced in 37 CFR 1.9(d), for purposes of paying reduced fees under section 41(a) and (b) of Title 35, United States Code, in that the number of employees of the concern, including those of its affiliates, does not exceed 500 persons. For purposes of this statement, (1) the number of employees of the business concern is the average over the previous fiscal year of the concern of the persons employed on a full-time, part-time or temporary basis during each of the pay periods of the fiscal year, and (2) concerns are affiliates of each other when either, directly or indirectly, one concern controls or has the power to control the other, or a third party or parties controls or has the power to control both.

I hereby declare that rights under contract or law have been conveyed to and remain with the small business concern identified above with regard to the invention, entitled Compositions and Methods for Treatment of Staphylococcal Infection While Suppressing Formation of Antibiotic-Resistant Strains by inventor(s) Michael Climo, et al.

described in

() the specification filed herewith Attorney Docket No; 7732-014-27

(XX) application serial no. _____, filed March 5, 1999,

() patent no. _____, issued _____.

If the rights held by the above identified small business concern are not exclusive, each individual, concern or organization having rights to the invention is listed below* and no rights to the invention are held by any person, other than the inventor, who would not qualify as an independent inventor under 37 CFR 1.9(c) if that person made the invention, or by any concern which would not qualify as a small business concern under 37 CFR 1.9(d) or a nonprofit organization under 37 CFR 1.9(e). *NOTE: Separate verified statements are required from each named person, concern or organization having rights to the invention averring to their status as small entities. (37 CFR 1.27)

NAME _____

ADDRESS _____

() INDIVIDUAL () SMALL BUSINESS CONCERN () NONPROFIT ORGANIZATION

NAME _____

ADDRESS _____

() INDIVIDUAL () SMALL BUSINESS CONCERN () NONPROFIT ORGANIZATION

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 CFR 1.28(b)).

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

NAME OF PERSON SIGNING Benjamin SpornTITLE OF PERSON OTHER THAN OWNER Vice-President, Legal SecretaryADDRESS OF PERSON SIGNING 4 Manhattanville Road
Purchase, New York 10577-2197SIGNATURE Benjamin Sporn DATE 4/5/99

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE APPLICATION OF: MICHAEL CLIMO, ET AL.

SERIAL NO: NEW DIVISIONAL APPLICATION

FILING DATE: HEREWITH

FOR: COMPOSITIONS AND METHODS FOR TREATMENT OF
STAPHYLOCOCCAL INFECTION WHILE SUPPRESSING
FORMATION OF ANTIBIOTIC-RESISTANT STRAINS

PRELIMINARY AMENDMENT

ASSISTANT COMMISSIONER FOR PATENTS
WASHINGTON, D.C. 20231

SIR:

Prior to examination on the merits, entry of the following amendments to the above-captioned patent application is respectfully requested.

IN THE CLAIMS:

Please cancel Claims 12-17.

Please amend Claim 1 as indicated below.

1. (Amended) A method of treating a staphylococcal infection in a mammal [in need of same, comprising administering an antimicrobial agent thereto] while suppressing the formation of antibiotic-resistant staphylococci [mutant] strains [resistant to said antimicrobial agent], said method comprising simultaneously administering [an amount of an anti-staphylococcal agent whose activity is mediated by cleavage of the glycine-containing cross-links of peptidoglycans of the cell wall of staphylococci (peptidoglycan active agent)] lysostaphin and [an amount of] another antibiotic effective against [sensitive] staphylococci[, wherein said] whose antibiotic activity is mediated by cell-wall activity (cell-wall active

antibiotic), [whereby the formation of staphylococci mutant strains resistant to said peptidoglycan active agent is suppressed,] wherein each of said [amount of peptidoglycan active agent] lysostaphin and [said amount of] said cell-wall active antibiotic are [each] present in amounts [individually sufficient to be therapeutically] effective [against] to therapeutically treat a sensitive [staphylococci] staphylococcal infection if each of said lysostaphin and said cell-wall active antibiotic are administered individually and wherein the amounts are combined such that said lysostaphin and said cell-wall active antibiotic, when co-administered, suppress the formation of staphylococcal strains resistant to said lysostaphin, said cell-wall active antibiotic and combinations of said lysostaphin and said cell-wall active antibiotic.

Please add new Claims 18-22.

--18. A method of enhancing the effectiveness of lysostaphin as a bacteriocin by suppressing formation of staphylococcal strains resistant thereto, comprising combining an amount of lysostaphin independently effective in therapeutically treating a staphylococcal infection in a mammal with an amount of a cell-wall active antibiotic sufficient to treat, independently, a staphylococcal infection in a mammal, wherein both the lysostaphin and the cell-wall active antibiotic are present in amounts which, when co-administered, suppress the formation of staphylococcal strains resistant to the lysostaphin, the cell-wall active antibiotic and combinations of lysostaphin and the cell-wall active antibiotic.

19. The method of Claim 18, wherein said cell-wall active antibiotic is a β -lactam or a glycopeptide.

20. The method of Claim 19, wherein said cell-wall active antibiotic is a β -lactam.

21. The method of Claim 20, wherein said β -lactam is selected from the group consisting of a penicillin, a cephalosporin and a carbapenem.

22. The method of Claim 21, wherein said β -lactam is penicillin.--

REMARKS

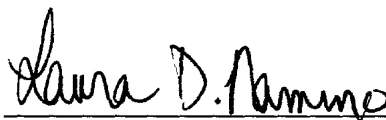
Applicants respectfully request that the Examiner consider the foregoing Preliminary Amendment upon initial consideration on the merits of the present application. Applicants submit that the Preliminary Amendment does not introduce new matter.

Applicants' claimed method treats a staphylococcal infection in a mammal while, at the same time, suppresses the formation of antibiotic-resistant staphylococci strains. In particular, by co-administering lysostaphin and a cell-wall active antibiotic according to Applicants' invention, the formation of strains of staphylococci resistant to the combination of lysostaphin and the cell-wall active antibiotic is suppressed. Furthermore, the cell-wall active antibiotic present suppresses the formation of new staphylococci strains that are resistant to lysostaphin.

Accordingly, Applicants submit that this application is in condition for examination and favorable consideration is respectfully requested.

Respectfully submitted,

PIPER MARBURY RUDNICK & WOLFE LLP



Steven B. Kelber
Registration No: 30,073
Attorney of Record

Laura D. Nammo
Registration No.: 42,024

1200 Nineteenth Street, N.W.
Washington, D.C. 20036-2412
Telephone No: (202) 861-3900
Facsimile No: (202) 223-2085

TITLE OF THE INVENTION

**COMPOSITIONS AND METHODS FOR TREATMENT
OF STAPHYLOCOCCAL INFECTION WHILE SUPPRESSING
FORMATION OF ANTIBIOTIC-RESISTANT STRAINS**

5

BACKGROUND OF THE INVENTION

Field of the Invention

This invention pertains to a method of treating staphylococcal infection in mammals, including humans. The method involves the simultaneous administration of a lysostaphin or other agent which attacks the glycine-containing peptide cross-links of the cell wall peptidoglycan found in staphylococci and an antibiotic, the antibiotic properties of which are mediated by its ability to affect the cell wall of the target staphylococci. This combined administration is effective in treating the staphylococcal infection, and at the same time suppresses the formation of strains resistant to lysostaphin or other peptidoglycan active agent.

15

BACKGROUND OF THE PRIOR ART

Lysostaphin is a bacteriocin secreted by a staphylococcal strain isolated and originally named *Staphylococcus staphylolyticus* (now *S. simulans*). The production of lysostaphin is described in U.S. Patent 3,278,378. Lysostaphin is an endopeptidase which cleaves the polyglycine cross-links of the peptidoglycan found in the cell walls of staphylococci. U.S. Patents 3,398,056 and 3,594,284 describe improvements to culture medium and inoculation techniques for the production of lysostaphin.

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The gene for lysostaphin from *S. simulans* has been sequenced and cloned, U.S. Patent 4,931,390. Lysostaphin for use as a laboratory reagent has been produced by fermentation of a non-pathogenic recombinant strain of *B. sphaericus*, from which it is readily purified. The cloning and sequencing of the lysostaphin gene permits the isolation of variant enzymes that have properties similar to or different from those of wild type lysostaphin. One such altered enzyme, bearing a single amino acid change, has been characterized and shown to have potent anti-staphylococcal activity both *in vitro* and in an animal infection model. U.S. Patent Application Serial No. 09/120,030, filed July 21, 1998 and incorporated herein by reference. Other lysostaphin analogues, including naturally occurring enzymes of this type have been established as potent agents capable of addressing difficult to treat bacterial diseases caused by staphylococcal infection. Other peptidases with related activity are known. Thus *lasA* protease and achromopeptidase, reported in Kessler, et al., J. Biol. Chem. 268:7503-08 (1993) and Li et al., J. Biochem. 122:772-778(1997), respectively, have anti-staphylococcal activity based on their digestion of glycine-containing cross-links in the peptidoglycan cell wall component. These agents may be used in this invention in place of lysostaphin.

The development of lysostaphin as an effective antibiotic to treat staphylococcal infection has been plagued, however, by a problem that is universal for antibiotic administration - the increasing development of antibiotic-resistant strains of mutant staphylococci. Already, a wide variety of staphylococcal infections resistant to various antibiotics that were previously the treatment of choice, including methicillin (methicillin resistant *S. aureus* are referred to as MRSA) and vancomycin-resistant strains (referred to as VISA) have been identified. Resistance to a wide variety of other antibiotics, not

exhibited by sensitive staphylococci, has been noted as well. MRSA, as well as strains resistant to other antibiotics, are discussed at length in Stranden, et al., J. Bacteriology 179(1):9-16 (1997). Further difficulties are encountered in that MRSA tend to accumulate a variety of other resistances as well. Multiresistant MRSA are typically treated with vancomycin, The Staphylococci In Human Diseases, 158-174 (Grossley, et al., editors 1997). Vancomycin itself may be toxic. Additionally, vancomycin resistance has recently been detected in staphylococci infections.

The problem posed by the continuing development of antibiotic-resistant infectious agents, such as staphylococci, is more than the difficulty involved in treating any individual patient. Popular press, as well as scientific journals, have noted the alarming increase in the generation of resistant strains, due in part to indiscriminate use or over-use of antibiotics. Each time an individual is treated with an antibiotic, whether needlessly or reasonably, the chance that a strain resistant to that particular treatment will arise is increased. Resistant strains of staphylococci have become endemic in many hospitals and pose a life-threatening danger to patients already debilitated by other ailments who become infected after admission to those hospitals.

Numerous articles have noted the development of resistance to either lysostaphin or β -lactams, such as methicillin, and the relationship there between. Thus, DeHart, et al., Applied Environmental Microbiology 61, 1475-1479 (1995) noted the development of mutant *S. aureus* recombinant cells that were resistant to lysostaphin, but susceptible to methicillin. Similar phenomenon are reported by Zygmunt, et al., Can. J. Microbio. 13,845-852 (1966), Polak, et al., Diagn. Microbiol. Infet. Dis. 17:265-270 (1993) and Dickson, et al., Yale J. Bio. Med. 41:62-67 (1968). Each of these references, as well as

later reports such as Ehlert, J. Bacteriology, 179:7573-7576 (1997) note that staphylococci that develop resistance to lysostaphin, either spontaneously or through induced recombination, become susceptible to methicillin treatment, and vice-versa. In all of these references, the uniform suggestion is to follow a course of administration of lysostaphin, even a short one, with administration of methicillin.

U.S. Patent 5,760,026, commonly assigned herewith, employs a specific method for treating mastitis, by intramammary infusion of lysostaphin. The patent reports, Table ID and elsewhere, that a synergistic result is predicted when combining lysostaphin and a β -lactam to treat mastitis, based on an *in vitro* assay. The bovine mastitis model is not predictive of *in vivo* administration of antibiotics, and the synergistic effects reported in U.S. Patent 5,760,026 have not been substantiated in an environment or model that would be reflective of *in vivo* administration to a mammal such as a human.

Those of skill in the art will be aware that there are a wide variety of staphylococcal strains. Many are resistant to conventional antibiotics, unlike sensitive strains. *S. aureus* strains are recognized as highly virulent and the most common single cause of serious systemic infections. Coagulase-negative staphylococcal species, although generally less invasive than *S. aureus*, are now responsible for a significant incidence of infections; particularly among debilitated or immunocompromised patients. As an example of such infection, one may point to endocarditis consequent to heart valve replacement. This is but one of a variety of intractable staphylococcal infections which are increasing due to the widespread use of antibiotics.

Accordingly, it remains an object of those of ordinary skill in the art to develop a method whereby even resistant staphylococcal infections in mammals, including humans,

may be effectively treated by the administration of antibiotics. Desirably, this method is developed so as suppress the formation of strains resistant to the antibiotics used.

SUMMARY OF THE INVENTION

The above goals, and others made clear by the discussions set forth below, are achieved by the simultaneous administration of an anti-staphylococcal agent, such as lysostaphin or other agent whose activity is mediated by cleavage of glycine-containing cross-links in the staphylococcal cell wall peptidoglycan and an antibiotic or antimicrobial agent whose activity is mediated by its ability to affect the cell wall of staphylococci. These cell-wall active agents include β -lactams and glycopeptides. Preferably, the cell-wall active antibiotic is a β -lactam.

There is no evidence of any synergistic effect achieved through the simultaneous administration of an anti-staphylococcal agent whose activity is mediated by cleavage of glycine-containing cross-links and a cell-wall active antibiotic in a model, *in vitro* or *in vivo*, that is predictive of benefit for *in vivo* administration of antibiotics in a mammal.

Indeed, those of ordinary skill in the art will recognize that for resistant staphylococci, such as MRSA, the administration of methicillin is not therapeutically effective in any amount. Surprisingly, Applicants have discovered that the combined administration of an anti-staphylococcal agent whose activity is mediated by cleavage of glycine-containing cross-links such as lysostaphin and the cell-wall active antibiotic not only effectively treats the infection, but suppresses the formation of staphylococci having resistance to the anti-staphylococcal agent whose activity is mediated by cleavage of glycine-containing cross-links.

While Applicants do not wish to be bound by this explanation, it appears that the spontaneous mutation commonly effective in conferring lysostaphin resistance in staphylococci renders the same highly susceptible to a cell-wall active antibiotic, such as methicillin. This is true even where the organism starts out as methicillin resistant.

5 Simultaneous administration of both appears to be uniformly effective in simultaneously eradicating the infection and suppressing the generation of new resistant strains.

Specifically, anti-staphylococcal agents like lysostaphin cleave glycine-containing cross-links. The mutation conferring resistance to this attack renders previously resistant strains sensitive to cell wall active antibiotics.

DETAILED DESCRIPTION OF THE INVENTION

This invention involves the administration of a pharmaceutical composition effective in the treatment of staphylococcal infection, which composition comprises at least two active agents, one an agent like lysostaphin which cleaves the glycine-containing cross-links of the cell wall peptidoglycans of staphylococci, the other a cell-wall active antibiotic. By

15 lysostaphin it is intended to refer herein to any enzyme, including lysostaphin wild type, a mutant or variant, or any recombinant or related enzyme that retains proteolytic activity against glycine-containing cross-links in the cell wall peptidoglycan of staphylococci. Variants may be generated by post-translational processing of the protein(either by enzymes present in a producer strain or by means of enzymes or reagents

20 introduced at any stage of the process) or by mutation of the structural gene. Mutations may include site-deletion, insertion, domain removal and replacement mutations. They may be recombinantly expressed, or otherwise. Other anti-staphylococcal active agents

acting by cleavage of the glycine-containing peptidoglycan cross-links include *lasA* protease and achromopeptidase. Such anti-staphylococcal agents which affect the peptidoglycan cross-links are embraced by the invention, but exemplified herein by reference to lysostaphin.

5 Cell-wall active antibiotics include β -lactams and glycopeptides. β -lactams are preferred. Suitable β -lactams include, but are not limited to, penicillins, such as penicillin, nafcillin, oxacillin, methicillin, amoxicillin and cloxacillin. Other β -lactams include cephalosporins and carbapenems. Representative cephalosporins include cephalothin, cefazolin, cefamandole, ceftazidime and others. Suitable carbapenems include imepenem and meropenem.

Suitable glycopeptides include vancomycin, teicoplanin and ramoplanin.

10 These two agents can be combined with further agents, adjuvants and the like, but are effectively administered in a pharmaceutically acceptable carrier. Administration is typically systemic, and may be intravenous (IV), intramuscular (IM), subcutaneous (SC), intraperitoneal (IP), intrathecal or topical. No synergistic effect of combining lysostaphin and a β -lactam or glycopeptide or cell-wall active antibiotic has been noted in a model predictive of *in vivo* mammalian administration. Accordingly, each agent of the effective combination must be administered in a therapeutically effective amount. It is to be noted, in this regard, that the amount to be administered is that which is therapeutically effective
15 when the lysostaphin and cell-wall active agent are administered together. Those of skill in the art will of course recognize that there is no therapeutically effective amount for, e.g., methicillin if the staphylococcal infection is an MRSA infection. Nonetheless, administration of therapeutic amounts of methicillin as determined against non-MRSA,
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combined with an amount of lysostaphin effective against staphylococci that are not lysostaphin-resistant will effectively treat staphylococcal infections even where the infection is resistant to one or other antibiotic. Accordingly, applicants have referred herein to "therapeutically effective amounts" to mean amounts effective to therapeutically treat sensitive *S. aureus* infection. This simultaneous administration, as opposed to sequential administration typified by the prior art, also surprisingly results in the suppression of strains resistant against either antibiotic, or their combination.

Any of a wide variety of pharmaceutically acceptable carriers and diluents, typically buffered, may be used. Appropriate pharmaceutical carriers are known to those of skill in the art. The formulations of this invention comprise a therapeutic amount of lysostaphin and a therapeutic amount of a cell-wall active antibiotic, such that when co-administered, the staphylococcal infection, either *S. aureus* or coagulase negative staphylococci, is treated, while the generation of resistant strains is suppressed. Other active agents that do not interfere with the activity of the two antibiotics may be co-administered.

Therapeutic values will range substantially given the nature of the staphylococcal infection, the individual, and the antibiotic being used in conjunction with lysostaphin. Representative values for anti-staphylococcal active agents such as lysostaphin, range from approximately 15-150 mg/kg body weight/day for human administration, with a preferred range of 25-100 mg/kg/day. Values for β -lactams based on nafcillin range from 50-250 mg/kg/day, with a preferred range of 100-200 mg/kg/day and glycopeptides like vancomycin are administered over a range of 10-75 mg/kg/day, with a preferred range of 15-50 mg/kg/day.

The administration course is not substantially different from that currently administered in single antibiotic treatments, and can range from 7-28 days, although typically, courses of 7-21 days are employed, and effective in treating a wide variety of staphylococcal infections.

EXAMPLES

To compare the development of resistant strains, growth curves for three methicillin resistant staphylococcal strains were obtained for *in vitro* growth in Mueller Hinton Broth.

Growth curves were completed in Mueller Hinton Broth (50 ml) in glass erlenmeyer flasks. Flasks were inoculated with 100 μ l of an overnight growth adjusted to 0.5 Macfarland to yield a starting concentration of approximately $10^5 - 10^6$ CFU/ml. Growth curves were done in the presence of lysostaphin, lysostaphin and oxacillin (1 μ g/ml) or no antibiotics (controls). Absorbance at OD 600 was recorded at 0, 2, 4, 6 and 24 hours. At 24 hours flasks were plated on MHA, MHA with lysostaphin (6 μ g/ml) and MHA with oxacillin (6 μ g/ml) in order to screen for resistant mutants. Three methicillin resistant *Staphylococcus aureus* strains were tested: 272855, 450M and Mu3.

Growth following 24 hour incubation with lysostaphin (0.0625 μ g/ml), lysostaphin (0.0625 μ g/ml) and oxacillin (1 μ g/ml), and no antibiotics, was recorded.

The data generated led to the following conclusions:

1. The addition of oxacillin to lysostaphin led to significant suppression of growth for all three strains.
2. The presence of oxacillin suppressed the expression of lysostaphin resistance among all three strains.

In order to demonstrate the effectiveness of the claimed invention, certain experiments were conducted. Checkerboard susceptibility testing was conducted to determine whether simultaneous administration of lysostaphin and oxacillin (a β -lactam) would be effective in suppressing the development of resistance. Oxacillin concentrations varied between 0.0156 μ g/ml and 1 μ g/ml. Lysostaphin concentrations varied between 0.00048 and 0.9 μ g/ml. Four strains were tested for evidence of synergy between lysostaphin and oxacillin; 27619, Col, 27227 and VA348. There was no evidence of synergy or antagonism over the concentration range tested. The MIC of lysostaphin was unchanged in the presence of oxacillin in concentrations up to 1 μ g/ml for all strains tested. The overnight growth of strains in the presence of lysostaphin and oxacillin was examined. Four strains were grown overnight in drug free media (MHB), MHB with 0.1 μ g/ml of lysostaphin, MHB with lysostaphin 0.1 μ g/ml and oxacillin 1 μ g/ml, and MHB with oxacillin 1 μ g/ml. The four strains tested included 450M, Col, and their lysostaphin resistant mutants 450 M lyso and Col lyso. The results are reflected in Table 1.

TABLE 1

Isolates	Growth in the presence of			
	MHB	lysostaphin 0.1 μ g/ml	oxacillin μ g/ml	Lyso + oxacillin
450M	+	+	+	-
450M lyso	+	+	-	-
Col	+	+	+	-
Col lyso	+	+	-	-

The same unpredicted result has been demonstrated through *in vivo* experiments based on the widely accepted rabbit model of aortic valve endocarditis, predictive of *in vivo*

administration to humans. When administered to staphylococcal infected rabbits at low doses (1 mg/kg bid, as compared with a minimum value of 5 mg/kg tid for sterilization) lysostaphin, as representative of anti-staphylococcal agents acting by cleavage of the glycine-containing cross-links, resulted in recovery of a number of resistant colonies, with high counts in vegetations and kidneys, while the same dosage together with nafcillin (a β -lactam) gave sterile kidneys, some sterile vegetations, and no resistant strains recovered. The simultaneous treatment of staphylococcal infection with suppression of resistant strain formation is an exciting and widely useful invention nowhere predicted in the art. This invention offers the possibility of treating staphylococcal infections while suppressing the generation of strains resistant to any or all active agents administered.

The inventive compositions and methods of this application have been disclosed generically, and by reference to specific example, examples are not intended to be limiting unless so indicated, and variations will occur to those of ordinary skill in the art without the exercise of inventive faculty. In particular, variations in the identity of the cell-wall active antibiotic to be co-administered with an anti-staphylococcal agent acting by cleavage of the glycine-containing cross-links, as well as various recombinant and mutant variants thereof, carriers and concentrations will occur to those of skill in the art without the exercise of inventive faculty, and remain within the scope of the invention, unless specifically excluded by the claims set forth below.

WHAT IS CLAIMED IS:

1. A method of treating a staphylococcal infection in a mammal in need of same, comprising administering an antimicrobial agent thereto while suppressing the formation of staphylococci mutant strains resistant to said antimicrobial agent, said method comprising
5 simultaneously administering an amount of an anti-staphylococcal agent whose activity is mediated by cleavage of the glycine-containing cross-links of peptidoglycans of the cell wall of staphylococci (peptidoglycan active agent) and an amount of another antibiotic effective against sensitive staphylococci, wherein said antibiotic activity is mediated by cell-wall activity (cell wall active antibiotic), whereby the formation of staphylococci mutant strains
10 resistant to said peptidoglycan active agent is suppressed, wherein said amount of peptidoglycan active agent and said amount of said cell-wall active antibiotic are each individually sufficient to be therapeutically effective against sensitive staphylococci, when co-administered.

2. The method of Claim 1, wherein said peptidoglycan active agent is lysostaphin

3. The method of Claim 1, wherein said administration is achieved through any one or more of intravenous (IV), intramuscular (IM), subcutaneous (SC), intraperitoneal (IP), intrathecal or topical administration.

4. The method of Claim 3, wherein said administration is SC, IP, intrathecal or topical.

5. The method of Claim 3, wherein said administration is either IV or IM.

6. The method of Claim 1, wherein said cell wall active antibiotic is a β -lactam or a glycopeptide.

7. The method of Claim 6, wherein said cell-wall active antibiotic is a β -lactam.

8. The method of Claim 7, wherein said β -lactam is selected from the group consisting of a penicillin, a cephalosporin and a carbapenem.

9. The method of Claim 8, wherein said β -lactam is a penicillin.

10. The method of Claim 1, wherein said staphylococcal infection is mediated by at least one *S. aureus* microorganism.

11. The method of Claim 1, wherein said staphylococcal infection is mediated by at least one coagulase-negative staphylococcal microorganism.

12. A composition effective in the treatment of staphylococcal infection in a mammal, comprising, as active agents, an anti-staphylococcal agent whose activity is mediated by cleavage of the glycine-containing cross-links of peptidoglycans of the cell wall of staphylococci (a peptidoglycan active agent) and an amount of another antibiotic effective against sensitive staphylococci, wherein said antibiotic activity is mediated by cell wall activity (a cell wall active antibiotic), further comprising a pharmaceutically acceptable carrier, wherein each of said peptidoglycan active agent and cell-wall active antibiotic are individually present in amounts which are therapeutically effective in treating a sensitive staphylococcal infection.

13. The composition of Claim 12, wherein said anti-staphylococcal peptidoglycan active agent is lysostaphin.

14. The composition of Claim 12, wherein said cell-wall active antibiotic is a β -lactam or a glycopeptide.

15. The composition of Claim 14, wherein said cell-wall active antibiotic is a β -lactam.

16. The composition of Claim 15, wherein said β -lactam is selected from the group consisting of a penicillin, a cepalosporin and a carbapenem.

17. The composition of Claim 16, wherein said β -lactam is a penicillin.

ABSTRACT

Co-administration of a lysostaphin or other anti-staphylococcal agent which cleaves cross-links of peptidoglycans of staphylococci cell walls such as lysostaphin and an antibiotic effective against staphylococci due to antibiotic activity mediated by cell-wall activity is effective against staphylococcal infection, even staphylococci that may be resistant to one or other of lysostaphin or the cell-wall active antibiotic. Co-administration simultaneously suppresses the generation of antibiotic-resistant mutant strains. Effective cell-wall active antibiotics include β -lactams and glycopeptides.

Docket No.: 7732-014-27

Declaration, Power of Attorney and Petition

WE (I) the undersigned inventor(s), hereby declare(s) that:

My residence, post office address and citizenship are as stated below next to my name,

We (I) believe that we are (I am) the original, first, and joint (sole) inventor(s) of the subject matter which is claimed and for which a patent is sought on the invention entitled

Compositions and Methods For Treatment of Staphylococcal Infection While Suppressing Formation of Antibiotic-Resistant Strains

the specification of which

☐ is attached hereto.

☒ was filed on March 5, 1999

as Application Serial No. 09/263,776

and amended on _____

☐ was filed as PCT international application

Number _____

on _____

and was amended under PCT Article 19

on _____ (if applicable).

We (I) hereby state that we (I) have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

We (I) acknowledge the duty to disclose information known to be material to the patentability of this application as defined in Section 1.56 of Title 37 Code of Federal Regulations.

We (I) hereby claim foreign priority benefits under 35 U.S.C. §119(a)-(d) or §365(b) of any foreign application(s) for patent or inventor's certificate, or §365(a) of any PCT International application which designated at least one country other than the United States, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or PCT International application having a filing date before that of the application on which priority is claimed. Prior Foreign Application(s)

Application No.	Country	Day/Month/Year	Priority Claimed
_____	_____	_____	<input type="checkbox"/> Yes <input type="checkbox"/> No
_____	_____	_____	<input type="checkbox"/> Yes <input type="checkbox"/> No
_____	_____	_____	<input type="checkbox"/> Yes <input type="checkbox"/> No

We (I) hereby claim the benefit under Title 35, United States Code, §119(e) of any United States provisional application(s) listed below.

(Application Number)

(Filing Date)

(Application Number)

(Filing Date)

We (I) hereby claim the benefit under 35 U.S.C. §120 of any United States application(s), or §365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of 35 U.S.C. §112, I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR §1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application.

Application Serial No.

Filing Date

Status (pending, patented, abandoned)

_____	_____	_____
_____	_____	_____
_____	_____	_____
_____	_____	_____

And we (I) hereby appoint Steven B. Kelber, Reg. No. 30,073; Marc R. Labgold, Ph.D., Reg. No. 34,651; Sharon E. Crane, Ph.D., Reg. No. 36,113; Catherine Bax Richardson, Reg. No. 39,007 and Ron Myers, M.S., Reg. No. 43,825 as our (my) attorneys, with full powers of substitution and revocation, to prosecute this application and to transact all business in the Patent Office connected therewith; and we (I) hereby request that all correspondence regarding this application be sent to Steven B. Kelber of Long Aldridge & Norman LLP, Attorneys At Law, 6th Floor, 701 Pennsylvania Avenue, N.W., Washington, D.C. 20004.

We (I) declare that all statements made herein of our (my) own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Michael CLIMO

NAME OF FIRST INVENTOR

Residence: 1802 Stonecrest Court

Richmond, Virginia 23236



Signature of Inventor

Citizen of: United States of America

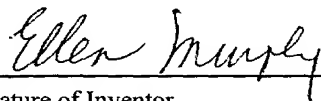
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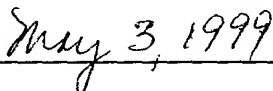
Date

Ellen MURPHY

NAME OF SECOND JOINT INVENTOR



Signature of Inventor



Date

Residence: 185 Beach Street

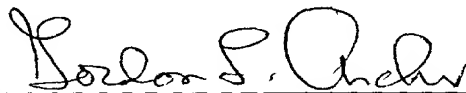
Bronx, New York 10464

Citizen of: United States of America

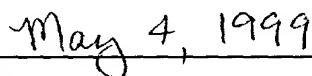
Post Office Address: Same As Above

Gordon ARCHER

NAME OF THIRD JOINT INVENTOR



Signature of Inventor



Date

Residence: 1504 Hearthglow Lane

Richmond, Virginia 23233

Citizen of: United States of America

Post Office Address: Same As Above

NAME OF FOURTH JOINT INVENTOR

Signature of Inventor

Date

Residence:

Citizen of:

Post Office Address: Same As Above

NAME OF FIFTH JOINT INVENTOR

Signature of Inventor

Date

Residence:

Citizen of:

Post Office Address: Same As Above

Residence: